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EXAMINER

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PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 10/717,993	Applicant(s) LIU ET AL.	
	Examiner MD. YOUNUS MEAH	Art Unit 1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 03 June 2009.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-23, 129 and 130 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-11, 12-23, 129 and 130 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____ |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

Claims 1-23 and 129-130 are currently pending in the instant application.

In response to a previous office action, a non-final action, mailed on 03/04/2009, Applicants' on 06/03/09 amended claims 14 and 16-17.

Applicants' response of 06/03/09 is acknowledged. Claims 1-23 and 129-130 are under consideration. Applicants' arguments filed on 06/03/09 have been fully considered but they are found unpersuasive. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Objection

Claim 1 is objected for not having an "and" before *bacillus stearothermophilus*.

There should be an "and" before *bacillus stearothermophilus*. Appropriate correction is required.

Claim Rejection 35 U.S.C 112 2nd paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-23 and 129-130 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 and 2-23, 129-130 (dependent on claim 1) are rejected under 35 U.S.C. 112, second paragraph, as being indefinite in the recitation of the phrase in claim 1 "at least about" because the " at least about" is a relative term which renders the claim

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indefinite. About encompasses both above and below a reference point whereas at least means less than or equal to the reference point.

Claims 1 and 2-23, 129-130 (dependent on claim 1) are rejected under 35 U.S.C. 112, second paragraph, as being indefinite because the method is omitting essential steps. The method is to produce lactic acid. Where is the step where lactic acid is produced? There is nothing to indicate that lactic acid is made as a result of culturing the yeast cell.

Claim 5 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite in the recitation of the phrase in claim 5 "greater than about" because the "greater than about" is a relative term which renders the claim indefinite. About encompasses both above and below a reference point whereas greater than means above the reference point.

Claims 2-4 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite in the recitation of the phrase in claims 2-4 "less than about" because the "less than about" is a relative term which renders the claim indefinite. About encompasses both above and below a reference point whereas less than means below the reference point.

Claims 6-7 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite in the recitation of the phrase in claims 6-7 "between about" because the "between about" is a relative term which renders the claim indefinite. About encompasses both above and below a reference point whereas between means inside the reference point.

Claims 129-130 are rejected under 35 U.S.C. 112, second paragraph, because of the following reason: The phrase "approximating the lowest pH of the parent yeast strain culture at which the parent yeast strain will grow and produce lactic acid" makes the claim unclear. It is not clear to the examiner how "approximating" is being used here. Approximating can mean several things. Does it mean to match the lowest pH at which the parent yeast strain can grow? Does it mean to gradually lower the pH? If the latter is true, then it is suggested "reducing the pH in the culture until the lowest pH at which the parent yeast strain will grow and produce lactic acid is achieved" or similar. Correction is required.

Claims 130 is rejected under 35 U.S.C. 112, second paragraph, because it is unclear as to how one could remove an aliquot of the culture when the parent yeast strain ceases to grow when the step prior to that requires culturing at a pH which would allow growth of the parent yeast strain. If the culture is at a pH which allows growth, how could you be taking an aliquot of a culture that is no longer growing? Correction is required.

Claim Rejection - 35 U.S.C 103a

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1-7, 12-20, 22-23 and 129-130 were rejected under 35 U.S.C. 103(a) as being obvious over Rajgarhia *et al.* (US pat 7229805) in view of Lee *et al. et al.* (UK patent 2251864, 1995). This rejection is maintained as discussed at length in the previous office action and discussed it again below.

Claims 1 is directed to a method of producing lactic acid, comprising: performing selection on a parent yeast strain that contains an exogenous lactate dehydrogenate gene encoding the amino acid sequence of a lactate dehydrogenate protein of an organism selected from the group consisting *Lactobacillus plantarum*, *bovine*, *Lactobacillus casei*, *Bacillus megaterium*, *Rhizopus oryzae*, or *Bacillus stearothermophilus* that is capable of being expressed in the parent yeast strain, to yield an acid-tolerant (AT) yeast strain that is capable of growing in a minimal medium at a lower pH than the parent yeast strain; and culturing in a minimal medium the acid-tolerant (AT) yeast strain, wherein the AT yeast strain produces less than about 1 ppm ethanol, wherein the exogenous lactate dehydrogenase gene is capable of being expressed in the AT yeast strain, and wherein a protein resulting from the expression of the exogenous lactate dehydrogenase gene has lactate dehydrogenase activity.

Claims 2-4, 12-23 and 129-130 are directed to the method of claim 1 wherein said AT yeast strain is C₂ carbon source dependent (claim 16) or independent (claims 2-4), grows in minimum medium having a carbohydrate or glucose as carbon source and/or produce lactic acid at a pH range 3.5-2.3. in a aerobic batch culture. Claims 5-7

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are directed to the method of claim 1 wherein said AT yeast strain produces 50 g lactic acid/100 g glucose to up to 70 g lactic acid /100 g glucose.

Rajgarhia et al. teach various recombinant acid tolerant (AT) yeast strains such as *Kluyvermyces*, and *Candida* having deleted pyruvate decarboxylase (PDC, example 15) gene (deletion of PDC gene results no ethanol production, column 4, lines 20-35) expressing, through integration into the yeast chromosome or through a plasmid, various exogenous LDH genes, including those from *Rhizopus oryzae* and *Bacillus megaterium* (column 28, lines 40-60). Rajgarhia et al. teach that said yeast strain is capable of growing in minimal medium or C₂ independent medium (column 21, lines 20-28) of cell at low pH (~2, column 5 lines 25-31, pH 2.5, fig 7). Rajgarhia et al. teach said yeast strains could produce lactic acid in aerobic growth conditions (column 5 lines 18-24). Rajgarhia et al. also teach said strain could produce up to 90gm lactic acid /100gm of glucose (examples 15-16 and table 1) wherein glucose is only carbon source (claim 8, Rajgarhia et al.). Rajgarhia et al. teach variants of recombinant yeast strains expressing exogenous LDH genes with the highest specific productivity during the anaerobic phase can be found not only to produce lactic acid faster, but also achieve a higher concentration at a lower pH, than the variants with lower specific productivity (example 15, column 38, lines 30-35) However Rajgarhia et al. do not teach a method of selection for the most acid tolerant, AT, yeast strain from] a parent yeast strain expressing exogenous LDH gene.

It is well known in the art that production of lactic acid in a cultural medium drop the pH of the medium (Lee et al. page 3) and most of the lactic acid producing bacteria

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do not grow at lower pH (Lee et al. page 1). Yeast cells, on the other hand, are viable at low pH (Rajgarhia et al. column 1 lines 25-46). Lactic acid is an industrially important chemical and to increase the yield of lactic acid, a microbial environment that can tolerate low pH is desired. Selection of a microbial cell that grows at low pH is advantageous for increased production of lactic acid. Rajgarhia et al. teach variants of recombinant yeast strains expressing exogenous LDH genes with the highest specific productivity during the anaerobic phase can be found not only to produce lactic acid faster, but also achieve a higher concentration at a lower pH, than the variants with lower specific productivity (example 15, column 38, lines 30-35).

Lee *et al.* teach the method of selection of mutant *lactobacillus* cell which is viable at low pH and produce lactic acid at low pH, wherein a parent *lactobacillus* cell is cultured at various low pH values and selection is made for the mutant *lactobacillus* cell that is viable at a lower pH than the parent strain (pages 6-7 and claim 9 of Lee et al.).

Therefore, one of ordinary skill in prior art would have been **motivated** to use yeast strains of Rajgarhia *et al.* expressing exogenous LDH genes which show the highest specific productivity during the anaerobic phase, produce lactic acid faster and a higher concentration at a lower pH (example 15, column 38, lines 30-35), grow them at various lower pH values and in minimal medium using the methods as described in examples 8, 15 (column 35, lines 35-53 and column 38, lines 36-44) and select the viable yeast cells that produce lactic acid at the lowest pH using the selection procedure of Lee et al. One of ordinary skill in the art would have been motivated to do so because recombinant acid tolerant yeast strains expressing exogenous LDH

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genes are used for the production of lactic acid, which is an industrially useful chemical. One of ordinary skill in the art would have been also motivated to select the most acid tolerable yeast strain that produce the highest amount of lactic acid by growing an acid tolerant yeast strain and to lower the pH to select the most viable strain at the lowest pH because i) use of acid tolerant yeast strains expressing exogenous LDH gene for the efficient production of lactic acid is well known in the art, ii) it is easy to purify lactic acid from yeast media at low pH, and iii) a yeast strain that produces lactic acid at the lowest pH will produce the most lactic acid and would require less] purification steps (column 25, lines 20-50 of Rajgarhia et al.).

As such it would have been obvious to one of ordinary skill in the art to use [*Kluyvermyces* strains] of Rajgarhia et al's expressing (through integration into yeast chromosome or through a plasmid) the *Rhizopus oryzae* LDH gene and grow it at various low pH values [and] in minimal medium using the methods as described in examples 8, 15 (column 35, lines 35-53 and column 38, lines 36-44) and then make a selection of the most acid tolerant (AT) viable strain using the selection procedure of Lee et al.

The expectation of success is high, because Rajgarhia et al teach strains which can produce lactic acid and Lee *et al.* teach selection of acid-tolerant strains by gradually reducing pH in the medium.

Arguments and response

Applicants' argue, at pages 7-16 of their amendment of 6/13/09 that 1) Rajgarhia *et al.* teach how to obtain maximum yield of lactic acid by engineered yeast but do not

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teach acid tolerant (AT) yeast strain and the yeast strain of Rajgarhia et al does not produce lactic acid at lower pH than that of parent yeast strain; and Rajgarhia et al is silent about optimal pH for lactic acid production; 2) by combining Lee et al teaching with Rajgarhia et al's, one of ordinary skill in the art would not have reasonable expectation of success at arriving at the applicants invention. Applicant further argue that Lee et al do not teach selection of yeast strain. Therefore one of ordinary skill in the art would not combine Rajgarhia et al's with Lee et al.

Applicants' arguments filed on 6/13/09 have been fully considered, but they are found unpersuasive. Applicants argument that Rajgarhia et al do not teach acid tolerant (AT) yeast strain because Rajgarhia et al yeast strain does not produce lactic acid at lower pH than that of parent yeast strain is not convincing because Rajgarhia et al. teach variants of recombinant yeast strains expressing exogenous LDH genes with the highest specific productivity during the anaerobic phase can be found not only to produce lactic acid faster, but also **achieve a higher concentration at a lower pH,** than the variants with lower specific productivity (example 15, column 38, lines 30-35). **Therefore Rajgarhia et al teach Acid tolerant variants yeast strain (that produce lactic acid at pH about 2.3-2.5)** by virtue of the fact that when more acid is in the culture, as is the case when higher productivity is achieved, pH will decrease.

Regarding the argument that Rajgarhia et al is silent about optimal pH for lactic acid production and the assertion that prior arts do not suggest "Selection of microbial cell that grow at most low pH is advantageous for increased production of lactic acid", it is noted that **Rajgarhia et al (column 1 lines 60-67) teach**

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the advantages of using low pH and high temperature to grow yeast cells for the production of lactic acid. Regarding argument [that] one of ordinary skill in the art would not combine Lee et al, with Rajgarhia et al, as explained above, the teaching of Lee et al is used for its teaching of a selection procedure for acid tolerant strains (the selection procedure is universal, and it can be applied to any microbial strain). One of ordinary skill in art can use the selection procedure of Lee et al and apply to the yeast strain production method of Rajgaria et al (growing yeast at a cultural comprising minimal medium and various pHs, as low as about 2.5 and below, example 8 and 15) and select a yeast strain which is viable at most lower pH (Rajgarhia et al yeast strain viable and produce lactic acid at a pH about 2.5 (Fig 7, before selection).

Claim 8 remains rejected under 35 U.S.C. 103(a) as being unpatentable over Rajgarhia *et al.* (US pat 7229805) in view of Lee *et al.* (UK patent 2251864, 1995) as applied to claims 1-7, 12-20, 22-23 and 129-130 above, and further in view of House *et al.* (US2003/0228671). This rejection is maintained as discussed at length in the previous office action and discussed it again.

Claim 8 is directed to the method of claim 1 wherein said AT yeast strain produce less than 1 ppm of glycerol.

Rajgarhia *et al.* teach various recombinant acid tolerant (AT) yeast strains, i.e., *Kluyvermyces*, *Candida*, *etc*, having deleted pyruvate decarboxylase (PDC, example 15) gene (deletion of PDC gene results no ethanol production, column 4 lines 25-34 of Rajgarhia et al.) expressing, through integration into the yeast chromosome or through

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a plasmid, various exogenous LDH genes, including those from *Rhizopus oryzae* (column 28, lines 41-61). The teaching of Lee *et al.* are described above.

However Rajgarhia *et al.* do not teach AT yeast strain producing less than 1 ppm of glycerol.

House *et al.* (US2003/0228671) teach a method of producing lactic acid using recombinant acid tolerant yeast strains expressing exogenous LDH gene without producing any ethanol or glycerol (page 17, paragraph 0209).

As such it would have been obvious to one of ordinary skill in the art to use recombinant acid tolerant yeast strains of House *et al.* (US2003/0228671) expressing exogenous LDH genes and grow them] at different media and at different pHs as taught by Rajgarhia *et al.* (US pat 7229805) and select the most acid tolerant yeast strain using the selection procedure of Lee *et al* and use it for the efficient production of lactic acid without producing any ethanol or glycerol.

Arguments and response

Applicants' argument, at page 16 of their amendment of 6/13/09, against claim 8 have been fully considered, but they found unpersuasive, as explained above in the response against the argument for claims 1-7, 12-20, 22-23 and 129-130.

Claim 21 was rejected under 35 U.S.C. 103(a) as being unpatentable over Rajgarhia *et al.* (US pat 7229805) in view of Lee *et al.* (UK patent 2251864, 1995) as applied to claims 1-7, 12-20, 22-23 and 129-130 above, and further in view of Rajgarhia

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et al. (US2004/0029256). This rejection is maintained as discussed at length in the previous office action and discussed it again.

Claim 21 is directed to the method of claim 1 wherein said AT yeast strain expresses an exogenous LDH gene from *lactobacillus plantarum*.

Rajgarhia *et al.* (US pat 7229805) teach various recombinant acid tolerant (AT) yeast strains such as *Kluyvermyces* and *Candida* having deleted pyruvate decarboxylase (PDC, example 15) gene (deletion of PDC gene results no ethanol production (column 4 lines 25-34) expressing, through integration into the yeast chromosome or through a plasmid, various exogenous LDH genes, including those from *Rhizopus oryzae* (column 28, lines 41-61). The teaching of Lee *et al.* are described above.

However Rajgarhia *et al.* (Pat 7229805) do not teach AT yeast strain expressing exogenous LDH gene from *lactobacillus plantarum*.

Rajgarhia *et al.* (US2004/0029256) teach recombinant acid tolerant yeast strains expressing exogenous LDH gene from *lactobacillus plantarum* (claim 10 of Rajgarhia *et al.* US2004/0029256).

As such, it would have been obvious to one of ordinary skill in the art to use recombinant acid tolerant yeast strains] of Rajgarhia *et al.* (US2004/0029256) expressing an exogenous *lactobacillus plantarum* LDH gene, grow it in different media and at different pHs as taught by Rajgarhia *et al.* (US pat 7229805) and [selecting the most acid resistant yeast strain using the selection procedure of Lee *et al.* and use it for the efficient production of lactic acid.

Arguments and response

Applicants' argument, at page 17 of their amendment of 6/13/09, against claim 11 have been fully considered, but they found unpersuasive, as explained above in the response against the argument for the claims 1-7, 12-20, 22-23 and 129-130.

Claims 9-10 were rejected under 35 U.S.C. 103(a) as being unpatentable over Rajgarhia et al. (US Pat 7229805) in view of Lee et al. (UK patent 2251864, 1995) as applied to claims 1-7, 12-20, 22-23 and 129-130 above, and further in view of Porro et al (US 7049108). This rejection is maintained as discussed at length in the previous office action and discussed it again.

Claims 9-10 are directed to the method of claim 1 wherein said AT yeast strain comprise *Saccharomyces* or *Saccharomyces cerevisiae*.

Rajgarhia et al. (US Pat 7229805) teach various recombinant acid tolerant (AT) yeast strains, i.e., *Kluyvermyces*, *Candida*, etc, having deleted pyruvate decarboxylase (PDC, example 15) gene (deletion of PDC gene results no ethanol production, (column 4 lines 24-35) expressing, through integration into the yeast chromosome or through a plasmid, various exogenous LDH genes, including those from *Bacillus megaterium* (column 28, lines 41-61). The teaching of Lee et al. are described above.

However Rajgarhia et al. (Pat 7229805) do not teach a *Saccharomyces* or *Saccharomyces cerevisiae* AT yeast strain.

Porro et al teach recombinant *Saccharomyces cerevisiae* yeast strain expressing various exogenous LDH genes including from *Bacillus megaterium*,

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wherein said yeast strain comprise deleted PDC genes so that it produce no ethanol. However Porro et al. do not teach a method of selection of AT yeast strain from the parent yeast strain expressing exogenous LDH gene.

As such, it would have been obvious to one of ordinary skill in the art to use recombinant acid tolerant *Saccharomyces cerevisiae* yeast strain of Porro et al]expressing an exogenous LDH gene from *Bacillus megaterium*, grow it in different media and pHs as taught by Rajgarhia et al. (US pat 7229805) and select the most acid resistant yeast strain using the selection procedure of Lee et al and use it for the efficient production of lactic acid.

Arguments and response

Applicants' argument, at page 17 of their amendment of 6/13/09, against claim 9 and 10 have been fully considered, but they found unpersuasive, as explained above in the response against the argument for claim 1-7, 12-20, 22-23 and 129-130.

Conclusion

Claims 1-23 and 129-130 are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Mohammad Meah whose telephone number is 571-272-1261. The examiner can normally be reached on 8:30-5PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's Andrew Wang can be reached on 571-272-0811. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should

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you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Mohammad Younus Meah
Examiner, Art Unit 1652

/Delia M. Ramirez/
Primary Examiner, Art Unit 1652